

RESEARCH NOTE

Emergence of a novel macrolide-resistant *Streptococcus pyogenes* *emm53* strain

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ABSTRACT

An unusual *emm53*, T-28/T-non-typeable, iMLSB phenotype clone represented a substantial proportion (28.6%) of invasive erythromycin-resistant group A streptococcus (GAS) isolates in the Czech Republic during 2003. Clonal analysis of *emm53* isolates between 2001 and 2004 revealed four pulsed-field gel electrophoresis (PFGE) patterns and two *emm* subtypes. Isolates produced identical PFGE patterns regardless of their invasiveness and/or tetracycline resistance. Multilocus sequence typing classified all isolates as ST340. An ST5 (*emm83*) isolate, a potential ancestor of ST340, was isolated in the Czech Republic from an impetigo patient in 1988. The Czech *emm53*/ST340 isolates shared only three of seven alleles with the original Lancefield *emm53*/ST11 isolate.

Keywords Antibiotic resistance, *emm* type, group A streptococcus, multilocus sequence typing, pulsed-field gel electrophoresis, *Streptococcus pyogenes*

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During the period 2001–2005, 215 invasive isolates of group A streptococcus (GAS) (one per patient) were obtained from 34 hospitals throughout the Czech Republic, of which ten (4.7% of the total) belonged to type *emm53*. The highest proportion

of *emm53* isolates was detected in 2003 (six of 59 isolates; 10.2%). Most *emm53* isolates were resistant to erythromycin (MIC range 8–64 mg/L), and some were also resistant to tetracycline (MIC range 16–32 mg/L). The overall rate of macrolide resistance among GAS isolates from invasive disease, based on data from 2003 to 2005, was 16.6% (27 of 162). Four (28.6%) invasive macrolide-resistant isolates were type *emm53* during 2003. The prevalent *emm* types among all invasive GAS isolates in 2001–2005 from the Czech Republic were *emm1*, *emm81*, *emm28*, *emm53*, *emm3* and *emm66*, while *emm28*, *emm53*, *emm117*, *emm4* and *emm78* were the most common types among macrolide-resistant invasive GAS isolates.

The age of patients ranged from 11 days to 97 years (11–58 years for *emm53* isolates), and the disease presentation varied from bacteraemia or arthritis to more serious presentations, e.g., necrotising fasciitis or septic shock. Details of the isolates from 13 cases are summarised in Table 1. Details of the surveillance methods [1,2] and overall macrolide resistance trends [3] have been described previously. The *emm53* type accounted for 4.0% (ten of 248) of non-invasive isolates from patients who presented with sore throat or mild skin lesions during 2001–2002. Three randomly selected non-invasive *emm53* isolates were added to the invasive group for comparison.

Thus, in total, 13 epidemiologically unlinked *emm53* isolates were characterised by classical serotyping (T, SOF and M), *emm* sequence-based typing, pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST), tested for resistance to erythromycin and tetracycline, and subjected to MLS_B resistance phenotyping. T, SOF and M typing were performed by standard methods [4], while *emm* typing was performed according to Whatmore *et al.* [5], as described by Beall (<http://www.cdc.gov/ncidod/biotech/strep/protocols.htm>), using primer 1 (TATT (C/G)GCTTAGAAAATTAA) and primer 2 (GCAAGTTCCTCAGCTTGTTT), with *emmseq2* (TATTCGCTTAGAAAATTAAAAACAGG) as the sequencing primer. PFGE was performed by the rapid method of Benson and Ferrieri [6], with the following minor modifications: *Sma*I digestion for 3 h; 6.0 V/cm for 10–35 s; 14°C; 0.5 × TBE buffer extended range (Bio-Rad); total time of 20 h. Visual assignment of PFGE types was according to published recommendations [7]. MLST was carried out as described previously (<http://spyogenes.mlst.net>).

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Table 1. Characterisation of 13 *emm53* group A streptococcal isolates from the Czech Republic that were included in the study

Isolate number	Year/district of isolation	Invasiveness (source)	T type	<i>emm</i> subtype	PFGE pattern	Erythromycin	Tetracycline	Phenotype ^b
1	2001/BN	NI/erythema (skin)	28	53.6	A1	R	R	iMLS _B
2	2001/VY	I/shock, necrotising fasciitis (blood)	NT	53.6	A1	R	R	iMLS _B
3	2002/BN	NI/mild lesion (skin)	NT	53.6	A1	R	R	iMLS _B
4	2002/BV	NI/erythema (skin)	NT	53.6	A1	R	R	iMLS _B
5	2002/ST	I/arthritis (joint fluid)	28	53.6	A1	R	R	iMLS _B
6 ^a	2003/VY	I/bacteraemia (blood)	NT	53.2	A2	S	R	S
7 ^a	2003/HB	I/bacteraemia (blood)	28	53.2	A3	S	R	S
8 ^a	2003/KA	I/bacteraemia (blood)	NT	53.2	A1	R	S	iMLS _B
9 ^a	2003/TV	I/cellulitis (deep tissue)	NT	53.6	A4	R	R	iMLS _B
10 ^a	2003/KN	I/cellulitis (deep tissue)	NT	53.2	A1	R	S	iMLS _B
11 ^a	2003/KN	I/necrotising fasciitis (deep tissue)	NT	53.6	A1	R	R	iMLS _B
12 ^a	2004/PE	I/cellulitis (deep tissue)	NT	53.6	A4	R	R	iMLS _B
13 ^a	2004/CB	I/necrotising fasciitis (deep tissue)	NT	53.6	A4	R	R	iMLS _B

I, invasive; NI, non-invasive; NT, non-typeable; PFGE, pulsed-field gel electrophoresis; S, sensitive; R, resistant; iMLS_B, inducible macrolide resistance.

^aIsolates included in the Strep-EURO programme.

^bMacrolide resistance phenotype.

Resistance phenotyping was based on the standard double-disk diffusion method [8], while resistance to erythromycin and tetracycline was determined by MIC tests [9].

All 13 isolates studied were either T-28 or T-non-typeable, which is an unusual finding, as *emm53* is normally associated with the T-3/13/B3264 complex. All 13 isolates were also SOF-negative and M-non-typeable, but were divided by *emm* sequence-based analysis into two groups that differed from each other by a single base at position 22 (starting at the end of the signal sequence), i.e., G for *emm53.2* and A for *emm53.6*.

PFGE yielded a single type, with four subtypes differing by a maximum of four bands. Although the results suggested an association between *emm* type and PFGE profile at the clonal group level, no correlation was observed between distinct PFGE genotypes and *emm* subtypes. Identical profiles were observed for both invasive and non-invasive isolates; tetracycline-resistant and tetracycline-susceptible isolates also shared identical profiles. The characterisation of the 13 *emm53* isolates is summarised in Table 1.

All of the study isolates were classified as ST340. Based on an eBURST analysis of the worldwide GAS database (http://spyogenes.mlst.net/sql/eburst_all.asp), ST5 was proposed as a possible ancestor of the ST340 strain. ST5, ST103, ST205 and ST340 are the sole members of the same clonal complex, defined as sharing six of seven alleles. Using a less stringently defined eBURST group (sharing five of seven alleles), ST340 belongs to the same group, together with 50 other sequence types.

A similar increase in type *emm53* isolates among invasive GAS isolates was observed in Finland in 1998 [10]; however, macrolide resistance was not

tested in this previous study. One of the *emm53* variants described in the Finnish study corresponds to subtype *emm53.2* identified in the present study (T. Siljander, personal communication). A T-8/25/Imp19 ST340 isolate of unknown *emm* type was also found in Norway in 2002 (<http://spyogenes.mlst.net/>). Based on published data, the *emm53* type has not been detected to date among erythromycin-resistant isolates in other European countries [11–14]. It is not known whether the incidence of this type is locally specific.

An *emm83*-ST5 strain was isolated in the Czech Republic from a patient with impetigo in 1988, and this may be a possible ancestor of the ST340 strain; the most recently detected member of this strain was a non-invasive skin isolate in 2002, but no other *emm83* isolates were detected among invasive GAS isolates during 1994–2005. No *emm53* isolates were detected among invasive GAS isolates during 1994–2000 (total *n* = 108). The incidence of *emm53* isolates declined in 2004, compared with 2003, when *emm28* became the predominant type among erythromycin-resistant invasive isolates from the Czech Republic. No isolate of type *emm53* was observed among invasive isolates in 2005. Further analysis of both invasive and non-invasive streptococcal isolates is particularly important in view of the development of a safe and effective anti-streptococcal vaccine. More extensive and statistically valid characterisation studies are required.

This is the first report of macrolide-resistant *emm53* isolates. All the *emm53* GAS isolates from the Czech Republic belonged to the ST340 clone. eBURST analysis revealed that the *emm53*-ST340 clone from the Czech Republic is related more closely to *emm83*-ST5 isolates than to the

Lancefield collection *emm*53-ST11 isolate. The new subtypes and sequence types from this study have been submitted to the central worldwide database.

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RESEARCH NOTE

Climate and genotypes of *Pneumocystis jirovecii*

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ABSTRACT

This study explored whether seasonal and/or climatic factors influenced detection of specific genotypes of *Pneumocystis jirovecii*. Between 1989 and 2001, 155 isolates of *P. jirovecii* were obtained from patients undergoing bronchoscopic alveolar lavage. For each isolate, the month and climatic conditions were noted. Genotypes of *P. jirovecii* were distinguished by polymorphisms in the mitochondrial large-subunit rRNA gene. There were monthly and seasonal variations in the frequency of detection of mixed genotypes (p 0.018 and p 0.031, respectively) and genotype 2 (p 0.029 and p 0.086, respectively). There was no association between month/season and genotypes 1, 3 and 4, or between monthly temperature or rainfall and any genotype.

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